The *Drosophila* Secreted Protein Argos Regulates Signal Transduction in the Ras/MAPK Pathway

Katsuhiko Mikoshiba,†,‡,‖,** Yasuyoshi Nishida,†† and Hideyuki Okano*,‡,[1]

*Department of Molecular Neurobiology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan; †Department of Molecular Neurobiology, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108, Japan; ‡Center for TARA, University of Tsukuba, Tsukuba, Ibaraki 305, Japan; §Department of Microbiology, The Jikei University School of Medicine, Minato-ku, Tokyo 105, Japan; ¶Department of Biology, Faculty of Science, Kyushu University, Ropponmatsu, Fukuoka 810, Japan; ‖Molecular Neurobiology Laboratory, Institute of Physical and Chemical Research (RIKEN), Tsukuba Life Science Center, Tsukuba, Ibaraki 305, Japan; **Mikoshiba Calciosignal Net Project, Exploratory Research for Advanced Technology (ERATO), Meguro-ku, Tokyo 153, Japan and ††Department of Biology, School of Science, University of Nagoya, Nagoya, Aichi 464, Japan

The *Drosophila argos* gene encodes a secreted protein with an EGF motif which acts as an inhibitor of cellular differentiation in multiple developmental processes. To investigate the cellular pathways regulated by Argos, we screened for mutations which could modify the phenotype caused by overexpression of *argos*. We show that the effects of *argos* overexpression on the eye and wing vein development are suppressed by gain-of-function mutations of the MAPKK/D-MEK gene (*Dsor1/D-mek*) and the MAPK/ERK-A gene (*rolled*) and were enhanced by loss-of-function mutations of *Star*. Loss-of-function mutations in components of the Ras/MAPK signaling cascade act as dominant suppressors of the phenotype caused by the *argos* null mutation. A loss-of-function *argos* mutation enhanced the overproduction of R7 neurons caused by gain-of-function alleles of *Son of sevenless* and *Dsor1*. Conversely, overexpression of *argos* inhibited formation of the extra R7 cells that was caused by high-level MAPK/ERK-A activity. A phenotype of the *sev* ; *argos* double mutants revealed that *sev* is epistatic to *argos*. These results provide evidence that Argos negatively regulates signal transduction events in the Ras/MAPK cascade.

INTRODUCTION

Cell to cell signaling is an important process in the regulation of cellular proliferation and differentiation during the development of multicellular organisms. Elucidation of both inductive and suppressive mechanisms required for the control of these signals is one of the central goals of developmental biologists. Cell fate determination in several developmental processes is known to be induced by signal transduction activated by a family of receptor tyrosine kinases which respond to extracellular signals. In *Drosophila*, the receptor tyrosine kinase encoded by *torso* controls the development of terminal structures in the embryo (Klingler *et al.*, 1988; Sprenger *et al.*, 1989), and the receptor tyrosine kinase encoded by *sevenless* (*sev*) is required for the specification of the R7 photoreceptor cell fate in the developing eye (Tomlinson and Ready, 1986; Hafen *et al.*, 1987; Basler *et al.*, 1988; Bowtell *et al.*, 1988). The *Drosophila* homologue of the EGF receptor (DER) is required for multiple developmental processes including eye development (Baker and Rubin, 1989, 1992; Xu and Rubin, 1993), wing vein development (Diaz-Benjumea and Garcia-Bellido, 1990; Diaz-Benjumea and Hafen, 1994), and dorsoventral patterning of the follicular cells in the developing egg chamber

[1] To whom correspondence and reprint requests should be addressed at Department of Molecular Neurobiology, Institute of Basic Medical Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305, Japan. Fax: (+81) 298-53-6966. E-mail: okanouot@md.tsukuba.ac.jp.

0012-1606/96 $18.00

(Schüpbach, 1987). Genetic screening for dominant modifiers of *sev* mutations has identified components of signal transduction cascades (Simon *et al.*, 1991). They include *downstream of receptor kinases* (*drk*) encoding a SH3-SH2-SH3 adapter protein (Simon *et al.*, 1993; Olivier *et al.*, 1993), *Son of sevenless* (*Sos*) encoding a putative guanine-nucleotide releasing factor (Rogge *et al.*, 1991), and *Ras1* (Simon *et al.*, 1991). Drk, Sos, and Ras1 also function downstream of Torso and DER (Simon *et al.*, 1993; Doyle and Bishop, 1993). Gap1 acts as a negative regulator of receptor tyrosine kinase signaling by down-regulating the activity of the Ras1 protein (Gaul *et al.*, 1992). Activated Ras1 causes the activation of D-raf (Ambrosio *et al.*, 1989; Dickson *et al.*, 1992), which results in the phosphorylation of MAPKK (MEK) encoded by *Dsor1* (Tsuda *et al.*, 1993), also referred to as *D-mek* (Lu *et al.*, 1994; Hsu and Perrimon, 1995), and of MAPK (ERK-A) encoded by *rolled* (*rl*) (Biggs and Zipurski, 1992; Biggs *et al.*, 1994; Brunner *et al.*, 1994a). In addition, *spitz* (*spi*), *rhomboid* (*rho*), and *Star* (*S*), members of the *spitz* group of genes, interact with components of the Ras signaling cascade in the developing eye and wing vein (Sturtevant *et al.*, 1993; Heberlein *et al.*, 1993; Freeman, 1994a; Kolodkin *et al.*, 1994; Tio *et al.*, 1994). Although a number of components in this signal cascade have been identified, the extracellular mechanisms that negatively regulate signal transduction processes in normal development remain unknown.

Argos, a secreted protein with an EGF motif, acts as an inhibitor of cell recruitment in the developing eye, wing vein, and chordotonal organ (Freeman *et al.*, 1992; Kretzschmar *et al.*, 1992; Okano *et al.*, 1992; Freeman, 1994b; Brunner *et al.*, 1994b; Sawamoto *et al.*, 1994; Okabe *et al.*, 1996). Argos is also required for the projection of photoreceptor axons during optic lobe development (Brunner *et al.*, 1994b; Sawamoto *et al.*, 1996). Our previous studies suggested that Argos plays an opposite regulatory role to Rho (Sawamoto *et al.*, 1994) and S (Sawamoto *et al.*, 1996) in a common pathway. Recently, it has been suggested that Argos inhibits activation of the EGF receptor by Spitz, a *Drosophila* TGF-α homologue (Schweitzer *et al.*, 1995a). To further study Argos function and to identify other genes associated with Argos-induced regulation of cellular differentiation, we have attempted to isolate mutations which may modify the phenotype caused by overexpressed Argos. Here, we report that components of the Ras/MAPK cascade act downstream of Argos. Present results indicate that Argos functions as a diffusible inhibitor in the Ras/MAPK pathway, which may regulate signal transduction events involved in cellular differentiation.

MATERIALS AND METHODS

Fly stocks. *Canton-Special* was used as the wild-type strain. *argos*^{152} (a hypomorphic allele) and *argos*^{257} (a null allele) were generated by imprecise excision of the P[*lac-W*] element of the enhancer trap line, *argos*^{sty2}. *D-raf*^{1} (Nishida *et al.*, 1988), *hs-argos* (Sawamoto *et al.*, 1994), *Dsor1*^{Gp158} (Tsuda *et al.*, 1993), and *Dsor1*^{Su1} (Tsuda *et al.*, 1993) have been previously described. *S*^{X155} (Heberlein *et al.*, 1993) was obtained from G. M. Rubin. *sev*^{d2}, *sev*^{S11}, and *S*^{218} were from Y. Hiromi. *Ras1*^{e2F} was from D. Yamamoto. *rl*^{Su14} and *rl*^{Su23} are the gain-of-function alleles generated by Y. M. Lim., Y. H. Inoue, L. Tsuda, and Y.N. (manuscript in preparation). Molecular analysis of the mutation has revealed that *rl*^{Su23} is the same mutation as *rl*^{SEM} (Brunner *et al.*, 1994a). These mutants show similar phenotypes in eye and wing development. Supernumerary R7 cells and extra wing veins are observed in *rl*^{Su14} and *rl*^{Su23} flies, although the phenotype of *rl*^{Su23} was more severe than that of *rl*^{Su14} (Y. M. Lim, Y. H. Inoue, L. Tsuda, and Y.N., manuscript in preparation).

Genetics. Fly culture and crosses were performed according to standard procedures. For heat shock experiments, second instar larvae were collected in a vial with medium and repeatedly heat-shocked at 36°C for 1 hr with an interval of 25°C for 5 hr using a temperature-programmable incubator.

Immunohistochemistry. Anti-Elav antibody was obtained from The Developmental Studies Hybridoma Bank. Cy3-conjugated anti-mouse IgG antibody was purchased from Chemicon. Eye imaginal discs were fixed in 4% paraformaldehyde in PBS for 30 min. After rinsing in PBNT (10% normal goat serum, 0.3% Triton X-100 in PBS), discs were incubated in anti-Elav antibody (1:100 dilution in PBNT) at 4°C overnight. After rinsing in PBNT, discs were incubated in Cy3-conjugated anti-mouse IgG (1:500 dilution in PBNT) for 1 hr at room temperature. After washing in PBS, discs were cleared in 80% glycerol in PBS and analyzed with a Zeiss Axioscop.

Scanning electron microscopy. Adult flies were dehydrated in a graded acetone series and dried at 60°C for 1 hr. Mounted flies were sputter-coated with platinum and observed with a Hitachi S-100 scanning electron microscope.

Wing preparation. Adult wings were dissected in 1-butanol and mounted with Permount (Fisher). Photographs were taken with a Zeiss Axioscop.

Plastic sections of adult heads. Adult heads were fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.2) at 4°C overnight. After washing in 0.1 *M* phosphate buffer (pH 7.2), heads were postfixed in 1% OsO4 in the same buffer for 2 hr at 4°C and then dehydrated in a graded ethanol series. After clearing in propylene oxide, heads were embedded in PolyBed 812 and sectioned with an ultramicrotome. One micrometer sections were stained with toluidine blue and examined with a Zeiss Axioskop.

RESULTS

Identification of Mutations Which Modify the argos Overexpression Phenotype

In order to identify gene products that share the common signaling cascade with Argos, a number of mutants showing eye or wing phenotypes were examined to determine whether they showed genetic interactions with *hs-argos*. *hs-argos* transgenic flies have a severe rough eye phenotype due to the reduction of retinular cells (Sawamoto *et al.*, 1994; Fig. 1B), in contrast with the regular array of ommatidia in the wild-type eye (Fig. 1A). We found that the *hs-argos* phenotype was remarkably suppressed by *Dsor1*^{Su1}, a

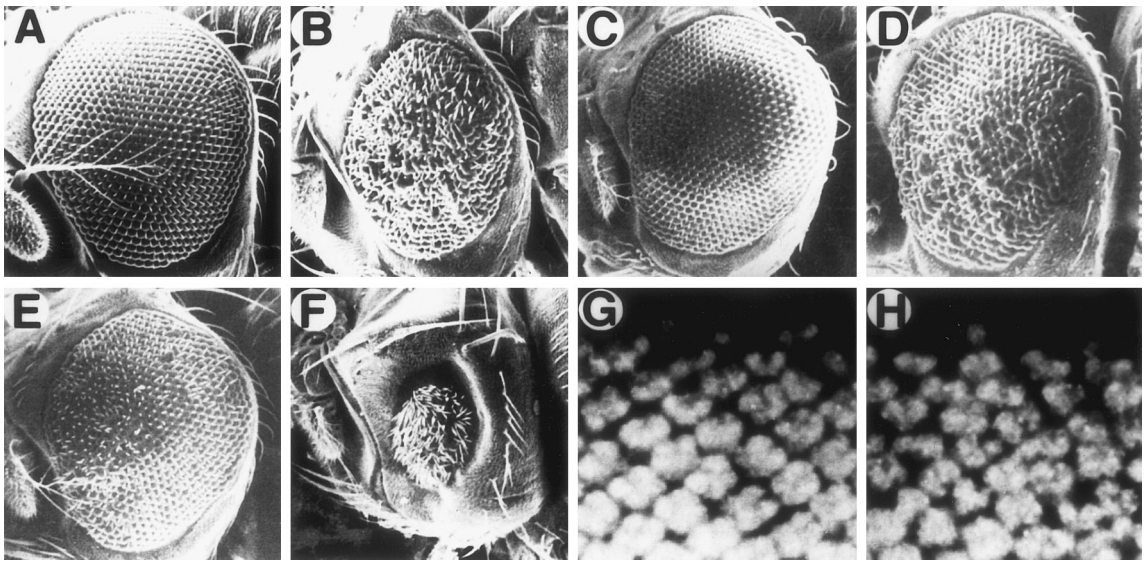

FIG. 1. Interaction of *hs-argos* with *Dsor1*, *rl* and *S* in eye development. (A–F) Scanning electron micrographs of adult eyes. (A) The wild-type eye shows a regular array of ommatidia. (B) *hs-argos*/+; *hs-argos*/+. Overexpression of *argos* causes a severe rough eye phenotype. (C) $Dsor1^{Su1}$/Y ; *hs-argos*/+; *hs-argos*/+. The external morphology of the eye is indistinguishable from that of the wild-type. (D) $Dsor1^{Su1}$/+ ; *hs-argos* /+ ; *hs-argos*/+. One copy of the gain-of-function *Dsor1* mutation considerably rescues the rough eye phenotype by *hs-argos* expression. (E) rl^{Su23}/*hs-argos*; *hs-argos*/+. One copy of the gain-of-function *rl* mutation also rescues the rough eye phenotype due to *hs-argos* expression. (F) S^{218}/*hs-argos*; *hs-argos*/+. The *hs-argos* phenotype is enhanced by a 50% reduction in the dose of the *S* gene. Neither S^{218} nor *hs-argos* alone causes the severe reduction in size of eyes like this. (G–H) Anti-Elav immuno staining of eye imaginal discs from third instar larva. (G) In the *hs-argos* /+ ; *hs-argos*/+, neuronal differentiation and ommatidial assembly appear normal. (H) S^{218}/*hs-argos*; *hs-argos*/+. Many ommatidia lack one or more photoreceptor cells.

gain-of-function allele of the *Dsor1* gene encoding a MAPKK (MEK) (Tsuda *et al.*, 1993), in a dose-dependent manner. The external morphology of the $Dsor1^{Su1}$ flies was normal (data not shown). The rough eye phenotype of *hs-argos* was almost completely suppressed in males carrying an X chromosome with the $Dsor1^{Su1}$ mutation (Fig. 1C), whereas females carrying one copy of the $Dsor1^{Su1}$ mutation showed a weak rough eye phenotype (Fig. 1D). The number and organization of the photoreceptor cells in the $Dsor1^{Su1}$/Y; *hs-argos* flies were indistinguishable from those of the wild-type (data not shown).

The phenotype caused by overexpression of *argos* was also suppressed by high-level MAPK activity. rl^{Su14} and rl^{Su23} are novel gain-of-function mutations of the gene encoding *Drosophila* MAPK (ERK-A) (Y.-M., Lim, Y. H., Inoue, L. Tsuda, and Y.N., manuscript in preparation). The eyes of rl^{Su23}/*hs-argos* ; *hs-argos*/+ flies had a less severe phenotype than those of *hs-argos* or rl^{Su23} flies (Fig. 1E). rl^{Su14} also suppressed the *hs-argos* phenotype (data not shown).

S is a member of the *spitz* group genes and known to interact with components of the Ras/MAPK cascade (Heberlein *et al.*, 1993; Kolodkin *et al.*, 1994). The gain-of-function phenotype of *argos* was enhanced by *S* mutations: weakly by the weak allele S^{X155} (Heberlein *et al.*, 1993) and more strongly by the null allele S^{218} (Kolodkin *et al.*, 1994). S^{218}/*hs-argos* ; *hs-argos*/+ flies had rougher and narrower eyes (Fig. 1F), when compared to *hs-argos*/+ ; *hs-argos*/+ flies (Fig.1B). To analyze this phenotype in more detail, we stained eye imaginal discs from S^{218}/*hs-argos* ; *hs-argos*/+ with anti-Elav antibody that recognizes neuronal nuclei. Normal expression of Elav was observed in the eye discs from the *hs-argos*/+ ; *hs-argos*/+ third instar larvae (Fig. 1G). However, the number of Elav-positive neuronal nuclei were decreased in the S^{218}/*hs-argos*; *hs-argos*/+ discs (Fig. 1H), indicating that the photoreceptor cells failed to undergo normal differentiation. Examination of tangential sections of the S^{218}/*hs-argos* ; *hs-argos*/+ adult eyes showed decreased numbers of photoreceptor cells in most of the ommatidia (data not shown).

We observed similar interactions of *hs-argos* with *Dsor1*, *rl*, and *S* in wing vein development. Extra veins were observed in 5% ($N = 106$) of $Dsor1^{Su1}$ wings (Fig. 2B), indicating a role for MAPKK in wing vein formation. This phenotype was similar to that of mutants in which DER signaling was activated, such as *Ellipse*, a gain-of-function *DER* mutation (Baker and Rubin, 1992), loss-of-function *Gap1* mutations (Gaul *et al.*, 1992), and gain-of-function mutations of *rl* (Brunner *et al.*, 1994a; Y.-M. Lim, Y. H. Inoue, L. Tsuda, and Y.N., paper in preparation). The similarities between these phenotypes imply that *Dsor1* functions downstream of DER as demonstrated by Hsu and Perrimon (1994). Heat-shocked *hs-argos*/+ ; *hs-argos*/+ adults had wings with

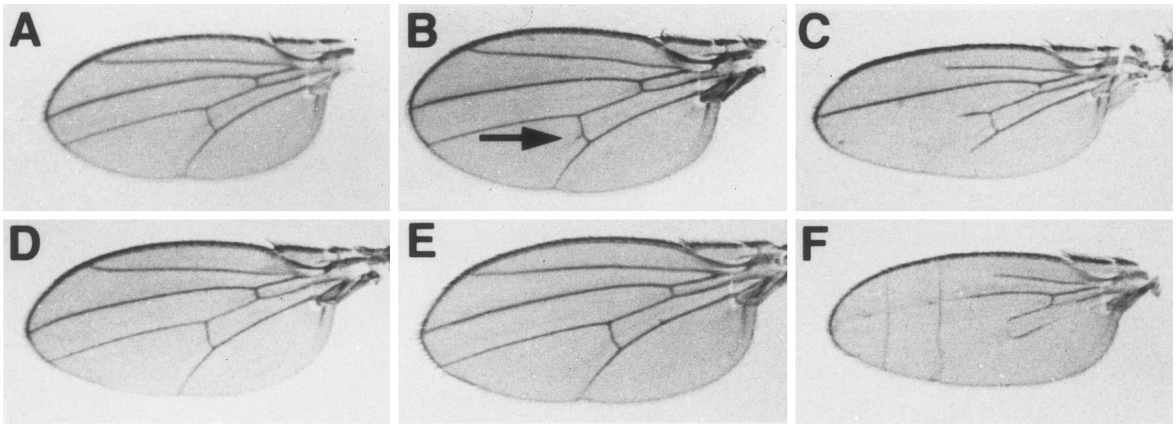

FIG. 2. Interaction of *hs-argos* with *Dsor1, rl,* and *S* in wing vein development. (A–F) Micrographs of adult wings. (A) Wild-type. (B) A wing of $Dsor1^{Su1}$/Y. Note the extra vein material between L4 and L5 crossing the posterior cross vein (arrow). (C) *hs-argos*/+ ; *hs-argos*/+. Longitudinal wing veins are shortened by *argos* overexpresion from the *hs-argos* transgene. This phenotype is completely suppressed by one copy of the gain-of-function mutations, $Dsor1^{Su1}$ (D) and rl^{Su23} (E). (F) A wing of *hs-argos*/S^{218} ; *hs-argos*/+ fly. A decrease in the dose of the *S* gene enhances the wing phenotype of *hs-argos*.

shortened longitudinal wing veins (Sawamoto *et al.,* 1994; Fig. 2C). This phenotype was completely suppressed by $Dsor1^{Su1}$ (Fig. 2D) and rl^{Su23} (Fig. 2E), and was enhanced by S^{218} (Fig. 2F) and S^{X155} (data not shown) in the same manner as was observed in eye development. Adult flies heterozygous for S^{218} and S^{X155} had normal wings.

These results indicate that Argos acts as a repressor in the Ras/MAPK cascade. We have also analyzed the genetic interactions between *argos* and other mutations affecting eye and/or wing vein development. The interaction of *argos* with the genes involved in the Notch pathways was examined, as Argos had been shown to contain an EGF motif and act as a lateral inhibitor in eye development (Freeman *et al.,* 1992; Kretzschmar *et al.,* 1992; Okano *et al.,* 1992; Freeman, 1994b; Brunner *et al.,* 1994b; Sawamoto *et al.,* 1994). However, there were no obvious interactions between *argos* and mutations in genes such as *Notch, Delta,* and *Serrate* (data not shown).

Decrease in Ras Signaling Activity Suppresses the argos Null Phenotype

To determine the epistatic relationship between *argos* and components in the Ras/MAPK cascade, we crossed loss-of-function alleles of *S* (S^{X155}), *Ras1* ($Ras1^{e2F}$), *D-raf* ($D\text{-}raf^{1}$), and *Dsor1* ($Dsor1^{Gp158}$) with a null allele of *argos*, $argos^{257}$ (Okabe *et al.,* 1996). If *argos* functions upstream of *S, Ras1, D-raf,* and *Dsor1,* and the *argos* phenotype is dose sensitive, a 50% reduction in the dose of these genes would be expected to suppress the *argos* phenotype. Flies heterozygous for $Ras1^{e2F}$, $D\text{-}raf^{1}$, or $Dsor1^{Gp158}$ had eyes that were indistinguishable from the wild-type eye (data not shown). In the $argos^{257}$ mutant eye, the regular array of ommatidia was disrupted and the posterior region showed characteristic blistering (Fig. 3A). However, a reduction by half in the dose of S^{X155}, $Ras1^{e2F}$, $D\text{-}raf^{1}$, or $Dsor1^{Gp158}$ resulted in considerable recovery of this phenotype. Fusion of lenses was seldom observed on the eyes of S^{X155}/+; $argos^{257}$/$argos^{257}$ flies (Fig. 3B), $Ras1^{e2F}$; $argos^{257}$/$argos^{257}$ flies (Fig. 3C), $D\text{-}raf^{1}$/*FM7*; $argos^{257}$/$argos^{257}$ flies (Fig. 3D), and $Dsor1^{Gp158}$/*FM7* ; $argos^{257}$/$argos^{257}$ flies (Fig. 3E). To further analyze the interactions between *argos* and *S, Ras1, D-raf,* or *Dsor1,* tangential sections of the compound eyes were examined. Most of the ommatidia in $argos^{257}$/$argos^{257}$ retinae contained extra outer photoreceptor cells (Fig. 3F). However, the number and organization of photoreceptor cells in the ommatidia appeared normal in S^{X155}/+; $argos^{257}$/$argos^{257}$ retinae (Fig. 3G). Formation of the extra outer photoreceptor cells by the $argos^{257}$ mutation was also considerably suppressed by halving the dose of $Ras1^{e2F}$ (Fig. 3H), $D\text{-}raf^{1}$ (Fig. 3I) or $Dsor1^{GP158}$ (Fig. 3J). In the $argos^{257}$ mutant ommatidia, the mean number of the extra outer photoreceptor cells was 1.3 (SD = 0.9, $N = 403$) (Fig. 4). In contrast, the mean number of extra photoreceptor cells decreased to 0.3 in $Ras1^{e2F}argos^{257}$/$argos^{257}$ (SD = 0.5, $N = 698$), to 0.3 in $D\text{-}raf^{1}$/*FM7*; $argos^{257}$/$argos^{257}$ (SD = 0.5, $N = 690$) and to 0.4 in $Dsor1^{Gp158}$/*FM7*; $argos^{257}$/$argos^{257}$ (SD = 0.6, $N = 965$) (Fig. 4). The differences in the mean number of the extra outer photoreceptor cells between the control and each of the other crosses in Fig. 4 were statistically significant (*t* test, $P < 0.001$). These results indicate that *argos* acts as a negative regulator of Ras-signaling and functions upstream of or in parallel with the Ras pathway.

Furthermore, we crossed *hs-argos* with flies carrying a constitutively active form of the *Ras1* transgene under control of the *sev* enhancer (*sev*E-$Ras1^{V12}$). In the *sev*E-$Ras1^{V12}$ eyes, there were multiple R7 in most of ommatidia (Fortini *et al.,* 1992). Overexpression of *argos* with the *hs-argos*

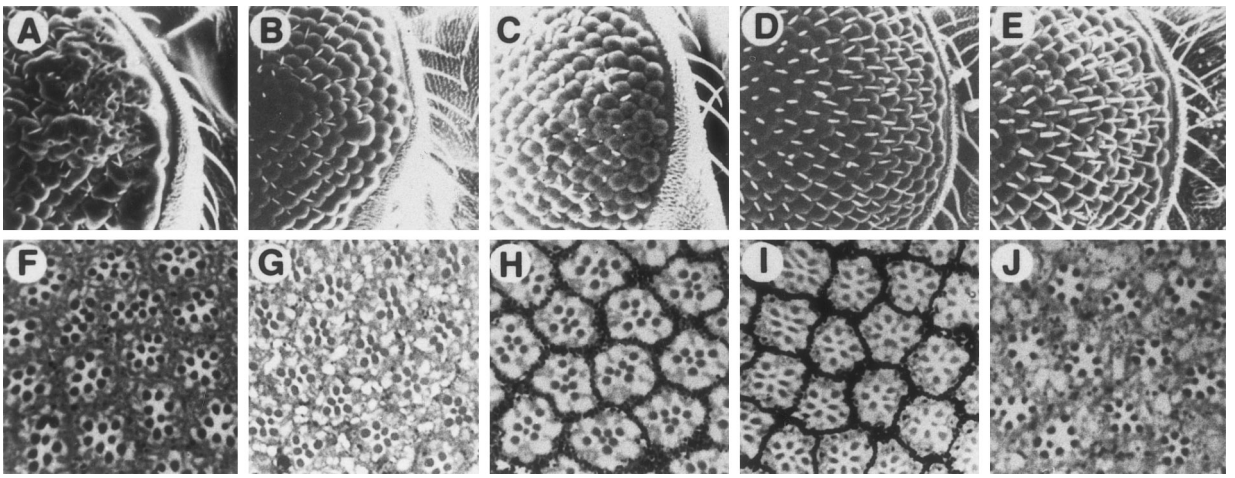

FIG. 3. Loss-of-function mutations of *S*, *Ras1*, *D-raf*, and *Dsor1* act as dominant suppressors of the *argos* null phenotype. (A–E) Scanning electron micrographs of adult eyes. (F–J) Sections of adult eyes. (A,F) $argos^{257}/argos^{257}$. (B,G) S^{X155}/CyO ; $argos^{257}/argos^{257}$. (C,H) $Ras1^{e2F-}$ $argos^{257}/argos^{257}$. (D,I) $D\text{-}raf^{1}/FM7a, B^{+}$; $argos^{257}/argos^{257}$. (E,J) $Dsor1^{Gp158}/FM7a, B^{+}$; $argos^{257}/argos^{257}$. In the *argos* mutant eye, the regular array of facets is disrupted and characteristic blistering are observed at the posterior region (A). Most of the ommatidia contain extra outer photoreceptor cells in the $argos^{257}/argos^{257}$ eye (E). These defects are dominantly suppressed by the loss-of-function mutations of *S* (B,G), *Ras1* (C,H), *D-raf*(D,I) or *Dsor1* (E,J). S^{X155} (Heberlein *et al.*, 1993), $Ras1^{e2F}$ (Simon *et al.*, 1991), $D\text{-}raf^{1}$ (Nishida *et al.*, 1988), and $Dsor1^{Gp158}$ (Tsuda *et al.*, 1993) were used as loss-of-function alleles. $argos^{257}$ (Okabe *et al.*, 1996) is a null allele generated by an imprecise excision of the P[*lacW*] insertion in the $argos^{sty2}$ enhancer trap line (Okano *et al.*, 1992).

transgene could not suppress the formation of multiple R7 by the constitutive activation of *Ras1* (data not shown). In addition, decrease of the gene dosage of *argos* could not affect the phenotype of *sev*E-$Ras1^{V12}$, i.e., the eyes of the *sev*E-$Ras1^{V12}$; $argos^{257}$/+ flies still contain extra R7 cells (data not shown). These results are consistent with our view that *argos* acts upstream of *Ras1*, as Ras signaling is constitutively activated by the expression of $Ras1^{V12}$ independently of the upstream signal.

Effects of argos on R7 Development

To further study the role of *argos* in Ras signaling-induced specification of photoreceptor cells, we crossed a weak loss-of-function allele of *argos* with gain-of-function alleles, $Dsor1^{Su1}$ (Tsuda *et al.*, 1993) and Sos^{JC2} (Rogge *et al.*, 1991). If Argos negatively regulates the Ras/MAPK cascade, a loss-of-function allele of *argos* is likely to enhance the gain-of-function alleles (signal dependent type) of the Ras/MAPK cascade.

Previous work showed that *Dsor1* which encodes a *Drosophila* MAPKK, also known as *D-mek* (Hsu and Perrimon, 1994), is required for development of terminal structures in embryonic development and acts downstream of *D-raf* in the *torso* pathway (Tsuda *et al.*, 1993). A temperature-sensitive mutation, $D\text{-}mek^{ts}$, produces a ''sevenless'' phenotype (Hsu and Perrimon, 1994), suggesting that *Dsor1* functions downstream of *sev*. To analyze the effect of the putative hyperactivation of MAPKK on eye development, the eyes of $Dsor1^{Su1}$ mutants were initially examined. The external morphology of the $Dsor1^{Su1}$ eye was indistinguishable from that of the wild-type. Analysis of sections through the compound eyes of $Dsor1^{Su1}$ adults revealed that supernumerary R7 cells had developed in about 2% ($N = 566$) of ommatidia (Fig. 5A), while other cell types were not affected. This indicates that the putative hyperactivation of MAPKK causes an overinduction of R7 cell fate. The formation of extra R7 cells depends on the upstream signal, as the sev^{d2} $Dsor1^{Su1}$ double mutant has a ''sevenless'' phenotype (Y. N., unpublished result).

To examine the effect of putative hyperactivation of MAPKK on the eye phenotype that results from a *argos* allele, a weak allele $argos^{152}$ was crossed with the $Dsor1^{Su1}$ mutant. Compound eyes of $argos^{152}$ homozygotes showed slightly rough morphology (data not shown). Analysis of sections through the $argos^{152}$ eyes revealed that about 15% ($N = 476$) of ommatidia contained extra outer (R1-6) photoreceptor cells, while R7 was not affected (Fig. 5B). $Dsor1^{Su1}$; $argos^{152}$ flies had eyes with a more severe roughness than that of either $Dsor1^{Su1}$ or $argos^{152}$ flies (data not shown). Surprisingly, sections of the eyes in the $Dsor1^{Su1}$; $argos^{152}$ flies showed that 25% ($N = 286$) of ommatidia contained extra R7-like cells (arrowheads), (Fig. 5C). In addition, $argos^{152}$ had a enhancing effect on the eye phenotype of a gain-of-function mutation of *Sos* (Sos^{JC2}) that was similar to the effect of $argos^{152}$ on $Dsor1^{Su1}$. Sos^{JC2} is also signal dependent allele and does not cause any visible abnormality in eye development (Rogge *et al.*, 1991). However, in the Sos^{JC2} ; $argos^{152}$ flies some of ommatidia contained extra R7-like cells (Fig. 5D). These data suggest that Argos may also in-

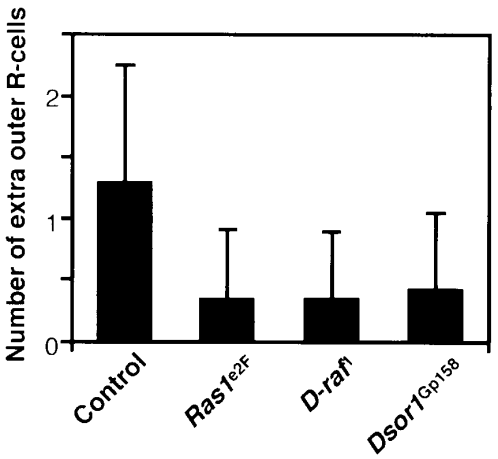

FIG. 4. Effects of gene activities of *Ras1*, *D-raf*, and *Dsor1* on the number of extra outer photoreceptor cells in the *argos* null eyes. The average number of extra outer photoreceptor-cells (R-cells) per ommatidium and the standard deviation were determined in tangential plastic sections of eyes of $argos^{257}/argos^{257}$ (control) animals or $argos^{257}/argos^{257}$ animals that are heterozygous for the indicated mutations. The mean number of extra outer photoreceptor cells in the control is distinct from those in *Ras1*, *D-raf*, and *Dsor1* with $P < 0.001$ as determined by *t* test.

hibit the overproduction of R7 cells by regulating the Ras/MAPK signaling.

To further study the role of *argos* in R7 formation, we crossed the *hs-argos* transgenic flies with a gain-of-function allele rl^{Su23}. Putative hyperactivation of MAPK resulted in the formation of multiple R7-like cells in the rl^{Su23} eyes (Fig. 5E). Formation of the extra R7 cells generated by rl^{Su23} was almost completely suppressed by *hs-argos* (Fig. 5F), further suggesting that Argos can influence the fate of R7 cells.

To clarify the relationship between *argos* and *sev*, we crossed null alleles of *sev* with $argos^{257}$. In the eyes of the *sev* null allele, sev^{d2}, all the ommatidia lack the R7 cells (Banerjee *et al.*, 1987). The null allele of *argos* failed to restore the production of R7 cells in the sev^{d2} mutant background (Fig. 6A). Therefore, loss of function of *argos* could not induce specification of R7 without the *sev* function. Furthermore, we examined the effect of overexpression of *argos* on the phenotype caused by activated Sev receptor. sev^{S11} (Basler *et al.*, 1991) is a constitutively active mutation encoding a mutant Sev protein that lacks the extracellular domain and causes a multiple R7 phenotype (Fig. 6B) similar to that of rl^{Su23}. Overexpression of *argos* from the *hs-argos* transgene did not inhibit the formation of multiple R7 by the constitutively activated receptor (Fig. 6C). These results of genetic interactions between *argos* and *sev* are discussed further in the Discussion.

DISCUSSION

Loss-of-function mutations in the *argos* gene cause an increase in cellular recruitment (Freeman *et al.*, 1992; Kretzschmar *et al.*, 1992; Okano *et al.*, 1992), whereas overexpression of *argos* inhibits differentiation (Freeman, 1994b; Brunner *et al.*, 1994b; Sawamoto *et al.*, 1994). These observations suggest that Argos regulates signal transduction events in cellular differentiation. The *argos* gene product is a secreted protein (Freeman, 1994b), its function is non-cell-autonomous (Freeman *et al.*, 1992; Kretzschmar *et al.*, 1992), and it may be referred to as a lateral inhibitor. In order to elucidate the signal cascade within which *argos* functions, the genetic interactions between *argos* and other mutations affecting eye and wing vein development were examined. A candidate for the cascade in which *argos* functions is the Ras/MAPK cascade. It is known that determination of the cell fate of photoreceptor cells and wing vein formation are induced by signal transduction events in the Ras/MAPK cascade. Therefore, we analyzed the genetic interactions of *argos* with components of the Ras/MAPK cascade. In addition, the genetic interactions between *argos* and components involved in the Notch signaling pathway, e.g., *Notch*, *Delta*, and *Serrate*, which are also involved in lateral inhibition and contain EGF-like repeats, were analyzed (Fehon *et al.*, 1990; Heitzler and Simpson, 1991; Rebay *et al.*, 1991). However, we could not detect any significant interactions between *argos* and components involved in the Notch signaling pathway. Therefore, Argos may be a lateral inhibitor which acts on the Ras/MAPK cascade rather than the Notch signaling pathway.

We have described previously that *argos* interacts with *rho* and *S* (Sawamoto *et al.*, 1994, 1996). *rho* and *S* are members of the *spitz* group and interact with components of the EGF receptor signaling pathway (Sturtevant *et al.*, 1993; Kolodkin *et al.*, 1994). The present study showed that *S* suppressed the phenotype caused by an *argos* null mutation and enhanced the phenotype caused by overexpression of *argos*. These strong interactions suggest that *argos* and *S* have opposing functions in a common pathway. It is known that Rho and S participate in the DER pathway (Sturtevant *et al.*, 1993; Kolodkin *et al.*, 1994). Schweitzer *et al.* (1995b) demonstrated that Rho and S may facilitate the processing of the Spitz protein, which is the TGF-α-like ligand for DER. Since *argos* interacts with both *rho* (Sawamoto *et al.*, 1994) and *S* (Sawamoto *et al.*, 1996; this work), *argos* is likely to act on the DER pathway.

In this work, *argos* was clearly shown to interact with major components of the signal pathway, acting downstream of the receptor tyrosine kinases such as *sev*, *DER*, and *torso*. The *argos* overexpression phenotype was suppressed by increased MAPKK (Dsor1) and MAPK (Rl) function (Figs. 1 and 2). On the other hand, the phenotype of a null allele of *argos* was suppressed by a decrease in Ras1, D-raf and Dsor1 function (Figs. 3 and 4). Therefore, *Ras1*, *D-raf*, and *Dsor1* act epistatic to *argos*. Moreover, the phenotype of hypomorphic alleles of *argos* was enhanced by gain-of-function alleles of *Sos* and *Dsor1* (Fig. 5). Based on this epistasis, the enhancement of the gain-of-function phenotypes of *Sos* and *Dsor1* by a loss-of-function allele of *argos* was likely to be due to the activation of Ras/MAPK signal-

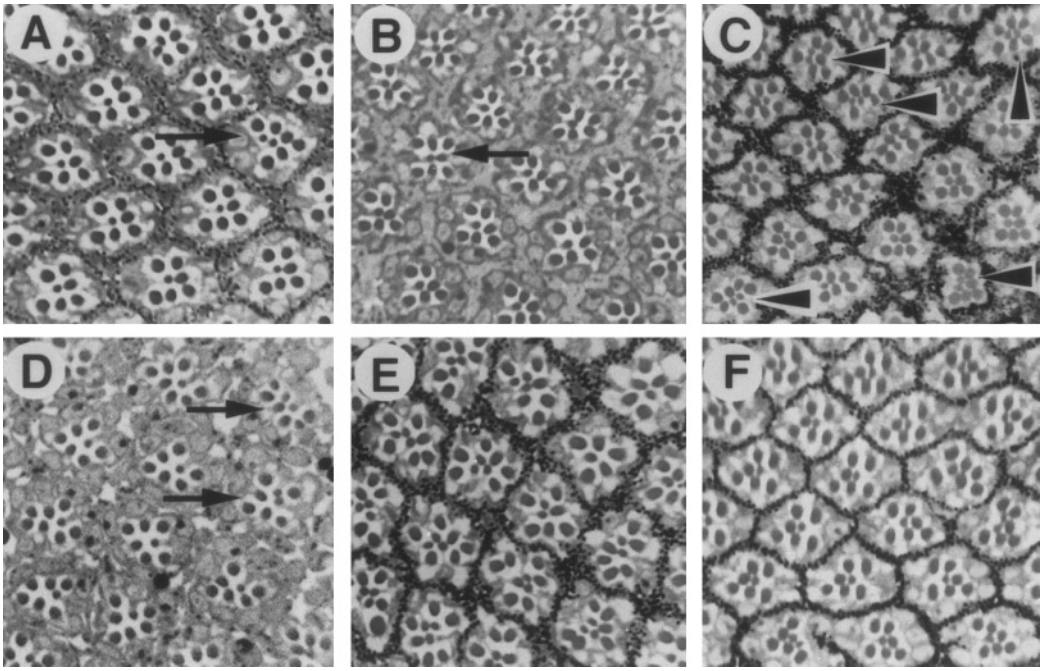

FIG. 5. Roles of *argos* in the formation of R7-like cells. (A–F) Sections of adult eyes. (A) *Dsor1*^{Su1}. Only 2% of the ommatidia have two R7-like cells (marked with an arrow). (B) *argos*^{152}/*argos*^{152}. The ommatidia with supernumerary outer photoreceptors are marked with an arrow. Note that R7 is not affected in this mutant. (C) *Dsor1*^{Su1}; *argos*^{152}/*argos*^{152}. About 25% of the ommatidia contained extra R7-like cells (arrowheads). (D) *Sos*^{JC2}; *argos*^{152}/*argos*^{152}. The ommatidia with extra R7 are marked with arrows. The formation of R7 cells is not affected by the *Sos*^{JC2} mutation alone. (E) *rl*^{Su23}. Almost all the ommatidia contain extra R7-like cells with a small rhabdomere. (F) Overexpression of *argos* from the *hs-argos* transgene eliminates most of the extra R7 cells formed by the *rl*^{Su23} mutation.

ing by decreased Argos function, rather than suppression of expression or function of Argos by activation of Ras/MAPK signaling. Therefore, these results strongly support the idea that the *argos* gene product is a negative regulator of signal transduction that acts upstream of the Ras/MAPK cascade.

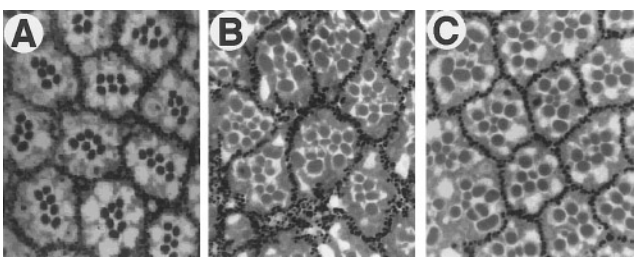

FIG. 6. Interaction between *argos* and *sev*. (A–C) Sections of adult eyes. (A) *sev*^{d2}/Y ; *argos*^{257}/*argos*^{257}. The ommatidia contained supernumerary outer photoreceptors but lack R7 cells. All the ommatidia lack R7 cells in the *sev*^{d2} eyes (Banerjee *et al.*, 1987). (B) *sev*^{S11}/+. Most of the ommatidia contain extra R7-like cells. (C) *sev*^{S11}/+ ; *hs-argos*/+ ; *hs-argos*/+. Overexpressed Argos cause a reduction of outer photoreceptor cells and pigment cells in number, but does not affect the formation of extra R7-like cells by *sev*^{S11}.

We showed that *argos* enhanced formation of the extra R7-like cells caused by the *Dsor1*^{Su1} and *Sos*^{JC2} mutations. We have not yet established the developmental origin of these R7-like cells. Previous studies showed that increased Sev signaling caused a transformation of mystery cells and cone cell precursors to R7 cells (Basler *et al.*, 1991; Fortini *et al.*, 1992). Therefore, loss of function of *argos* may make mystery cells and/or cone cell precursors transform to R7 cells by elevating the Ras/MAPK activities in the *Dsor1*^{Su1} ; *argos*^{152} and *Sos*^{JC2} ; *argos*^{152} mutants. We have also found that overexpressed *argos* suppressed the extra R7 formation in the *rl*^{Su23} allele, in which MAPK is hyperactivated in a signal-dependent manner (Y.N., unpublished result). These results imply that Argos functions in mystery cells and/or cone cell presursors to prevent their differentiation to the R7 phenotype by negatively regulating Ras/MAPK signaling. Another possible interpretation of these data is that the effect of Argos on the production of R7 cells is due to an earlier defect in the specification of the R8 cells. Although the loss-of-function mutation of *argos* does not affect the staining pattern of larval eye discs with a antibody against Boss (data not shown), we cannot rule out the possibility that the effect of Argos on R7 development is caused by a change in differentiation of R8. In this case, the most likely candidate for the Argos receptor is DER, since Spi/DER sig-

naling is required for this step (Freeman, 1994a; Tio *et al.*, 1994). The similar effects of *S* mutations on R7 production may also be interpreted as a secondary indirect consequence of weakened R8 specification (Kolodkin *et al.*, 1994).

Argos was shown to inhibit activation of the EGF receptor (DER) by Spi in a cell culture system (Schweitzer *et al.*, 1995a). This study also indicated a genetic interaction between *argos* and *DER* (Schweitzer *et al.*, 1995a). These results support the view that Argos functions as a competitive antagonist of DER. The effect of *argos* overexpression on R7 formation in rl^{Su23} (Fig. 5F) and R7 phenotypes in the $Dsor1^{Su1}$; $argos^{152}$ (Fig. 5C) and Sos^{JC2} ; $argos^{152}$ mutants (Fig. 5D) could be explained by the effect of Argos on DER, since both DER and Sev are likely to contribute to the activation of Ras in the cone cell precursors (Begemann *et al.*, 1995).

Alternatively, the regulatory mechanism of the number of R7 cells by Argos can be interpreted as follows. It is clear that Argos is not essential for the regulation of R7 cell specification, since an *argos* null mutation does not affect the number of R7 cells in ommatidia. However, we showed that ubiquitous overexpression of Argos could inhibit R7 production in the wild-type (Sawamoto *et al.*, 1994) as well as the production of extra R7 in rl^{Su23} (Fig. 5F). Furthermore, our present results indicated interesting genetic interactions between *argos* and *sev*, although previous data put more emphasis on the possibility that *argos* encodes a specific DER antagonist (Schweitzer *et al.*, 1995b). Here, we showed that *hs-argos* could not overcome a *sev* gain-of-function allele, sev^{S11} (i.e., the sev^{S11} ; *hs-argos* double mutants have extra R7 cells) and that combining null alleles of *argos* and *sev* gave the *sev* loss of R7 phenotype. These data are consistent with the suggestion that *sev* is epistatic to *argos*, implying that Argos might have broader specificity than previously suspected.

We also analyzed the genetic interactions between *argos* and *spitz* or *DER*, but failed to obtain any clear results showing significant interactions (data not shown). This may have been due to a difference in the conditions used for the heat-shock experiments and/or the mutant alleles tested. Therefore, the receptor for Argos remains unknown. There may be several mechanisms for Argos mediated inhibition of signal transduction by the receptor tyrosine kinases (Fig. 7). Since *argos* encodes an EGF-like ligand, it is possible that Argos binds directly to the EGF receptor, competing with Spi, and inhibits activation of the receptor. Alternatively, Argos may bind to another unknown receptor which may negatively control signaling from DER and Sev through the modulation of Ras/MAPK cascade, thereby setting an appropriate threshold for signal activation (the transmodulation model). In the latter case, the putative Argos receptor may activate intracellular factors such as protein phosphatases or GTPase activating proteins (GAPs) which negatively regulates the signal transduction. It is possible that Argos activates protein kinase C, as PKC is known to block signal transduction by the EGF receptor in mammals (Cochet *et al.*, 1984). Recently, Cullen *et al.* (1995) revealed that an

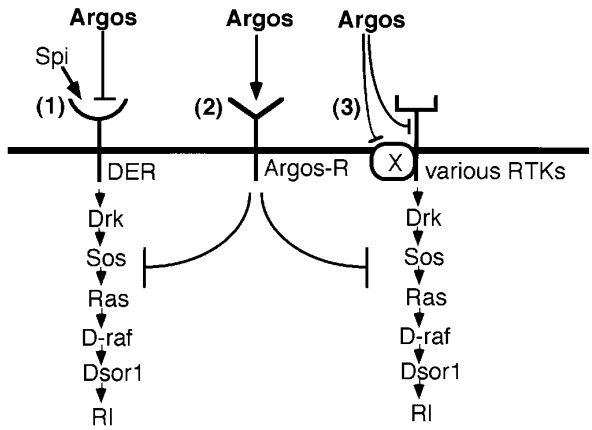

FIG. 7. Possible mechanisms for Argos inhibition of the Ras/MAPK signaling. The signal transduction for cellular differentiation is triggered by interaction between the TGF-α-like ligand (Spi) and the EGF receptor (DER). There is genetic evidence that suggests the involvement of other intracellular components (Ras1, D-raf, Dsor1, and Rolled), which act downstream of the receptor in a linear manner (reviewed by Dickson and Hafen, 1993). As Argos is a secreted protein with an EGF motif (Freeman, 1994b) and functions non-cell-autonomously (Freeman *et al.*, 1992; Ktetzchmar *et al.*, 1992), it is likely to function as a diffusible ligand. Argos functions as an inhibitor for cellular differentiation of photoreceptor cells, cone cells, pigment cells, wing veins, and chordotonal organs (Freeman, 1994b; Brunner *et al.*, 1994b; Sawamoto *et al.*, 1994; Okabe *et al.*, 1996). Argos is likely to act to inhibit transformation of cone cells and/or mystery cells to R7 cells. The results presented in this paper indicate that Argos inhibits the signal transduction of the Ras/MAPK cascade. The three major models for Argos inhibition of the Ras/MAPK signaling cascade are as follows. (1) Argos binds to DER. Consequently, Argos inhibits the activation of the receptor by competing with Spi. (2) Argos can bind to another specific receptor (Argos-R) which blocks signal transduction in the Ras/MAPK cascade (the transmodulation model). In this case, the unknown receptor may activate factors that negatively regulate signal transduction such as protein phosphatases or GTPase activating proteins. (3) Argos inhibits putative potentiating cofactors (indicated as ''X'') common to receptor tyrosine kinases (RTKs) or Argos can directly bind to the regions shared by various receptor molecules themselves, thereby generally inhibiting functions common to receptor tyrosine kinases.

Ins(1,3,4,5)P_4-binding protein has RasGAP activity and that this activity is specifically stimulated by binding to Ins(1,3,4,5)P_4. Consequently, Argos may activate inositol phosphate synthesis and then Gap1, which blocks Ras signaling.

In the transmodulation model, however, it is not clear why *hs-argos* could not overcome a dominant gain-of-function *sev* allele, sev^{S11}. Another possible model is that Argos interferes more generally with a function common to receptor tyrosine kinases. It is possible that there are shared potentiating cofactors (indicated as ''X'' in the figure) for these receptors which are inhibited by Argos or that Argos can

bind to regions shared by various receptor molecules themselves.

Genetic screening for modifiers of the *argos* phenotype may identify genes involved in the cascade required for the negative regulation of the inductive signals. *balge* and *soba* have been recently isolated in a screening for modifiers of *argos* as candidates for mutations of genes encoding the molecules involved in the signaling cascade for Argos (Wemmer and Klämbt, 1995).

Argos is the first example of a secreted protein that inhibits activation of receptor tyrosine kinase(s) and signal transduction in the Ras/MAPK cascade. Correct regulation of cellular differentiation may require diffusible inhibitor proteins such as Argos to regulate the levels of inductive signals from receptor tyrosine kinases. Components of the EGF receptor signaling cascades are highly conserved in evolution (Dickson and Hafen, 1994). Therefore, it is likely that there is a mammalian homologue of Argos. An Argos homologue may play key roles in the regulation of cellular differentiation and proliferation during mammalian development. Furthermore, if a human homologue of Argos is available, the inhibitory effect of Argos on the Ras/MAPK signaling may have application in the gene therapy of cancer.

ACKNOWLEDGMENTS

The authors thank Yasushi Hiromi, Eric Han, and Miyuki Yamamoto for valuable comments on the manuscript, Yoshitoshi Kasuya, Daisuke Yamamoto, Shingo Yoshikawa, Leo Tsuda, Lim Young-Mi, Masayuki Miura, and all members of Okano Laboratory at University of Tsukuba for valuable discussion, Keiko Kawamatsu, Shouko Aoki, and Sayaka Satoh for maintenance of fly strains, Naomi Sawamoto for her help in preparation of the manuscript, Noriko Sugae and Yoshiki Ohno for technical instruction, Daisuke Yamamoto, Yasushi Hiromi, Gerald M. Rubin, the Bloomington Stock Center, and the Indiana Stock Center for fly stocks, and Toshihiko Hosoya for anti-ELAV antibody. This work was supported by grants from the Ministry of Education, Science and Culture of Japan (to K.S. and H.O.) and from the TARA Okano project, center for TARA, University of Tsukuba (to H.O.). K.S. was supported by the Japan Society for the Promotion of Science.

REFERENCES

Ambrosio, L., Mahowald, A. P., and Perrimon, N. (1989). Requirement of the *Drosophila raf* homologue for *torso* function. *Nature* 342, 288–291.

Baker, N. E., and Rubin, G. M. (1989). Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor. *Nature* 340, 150–153.

Baker, N. E., and Rubin, G. M. (1992). *Ellipse* mutations in the *Drosophila* homologue of the EGF receptor affect pattern formation, cell division, and cell death in eye imaginal discs. *Dev. Biol.* 150, 381–396.

Banerjee, U., Renfraz, P. J., Pollock, J. A., and Benzer, S. (1987). Molecular characterization and expression of *sevenless*, a gene involved in neuronal pattern formation in the Drosophila eye. *Cell* 49, 281–291.

Basler, K., and Hafen, E. (1988). Control of photoreceptor cell fate by the sevenless protein requires a functional tyrosine kinase domain. *Cell* 54, 299–311.

Basler, K., Christen, B., and Hafen, E. (1991). Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing Drosophila eye. *Cell* 64, 1069–1082.

Begemann, G., Michon, A.-M., Voorn, L., Wepf, R., and Mlodzik, M. (1995). The *Drosophila* orphan nuclear receptor Seven-up requires the Ras pathway for its function in photoreceptor determination. *Development* 121, 225–235.

Biggs, W. H., and Zipursky, S. L. (1992). Primary structure, expression, and signal-dependent tyrosine phosphorylation of a *Drosophila* homolog of extracellular signal-regulated kinase. *Proc. Natl. Acad. Sci. USA* 89, 6295–6299.

Biggs, W. H., Zavitz, K. H., Dickson, B., Straten, A., Brunner, D., Hafen, E., and Zipursky, S. L. (1994). The *Drosophila rolled* locus encodes a MAP kinase required in the sevenless signal transduction pathway. *EMBO J.* 13, 1628–1635.

Bowtell, D. D. L., Simon, M. A., and Rubin, G. M. (1988). Nucleotide sequence and structure of the *sevenless* gene of *Drosophila* melanogaster. *Genes Dev.* 2, 620–634.

Brunner, D., Oeller, N., Szabad, J., Biggs, W. H., III, Zipursky, S. L., and Hafen, E. (1994a) A gain-of-function mutation in *Drosophila* MAP kinase activates multiple receptor tyrosine kinase signaling pathways *Cell* 76, 875–888.

Brunner, A., Twardzik, T., and Schneuwly, S. (1994b). The *Drosophila giant lens* gene plays a dual role in eye and optic lobe development: Inhibition of differentiation of ommatidial cells and interference in photoreceptor axon guidance. *Mech. Dev.* 48, 175–185.

Cochet, C., Gill, G. N., Meisenhelder, J., Cooper, J. A., and Hunter, T. (1984). C-kinase phosphorylates the epidermal growth factor receptor and reduces its epidermal growth factor-stimulated tyrosine protein kinase activity. *J. Biol. Chem.* 259, 2553–2558.

Cullen, P. J., Hsuan, J. J., Truong, O., Letcher, A. J., Jackson, T. R., Dawson, A. P., and Irvine, R. F. (1995). Identification of a specific Ins(1,3,4,5)P4-binding protein as a member of the GAP family. *Nature* 376, 527–530.

Diaz-Benjumea, F., and Galcia-Bellido, A. (1990). Behavior of cell mutant for an EGF receptor homologue of *Drosophila* in genetic mosaics. *Proc. R. Soc. London Biol. Sci.* 242, 36–44.

Diaz-Benjumea, F., and Hafen, E. (1994). The sevenless signaling cassette mediates *Drosophila* EGF receptor function during epidermal development. *Development* 120, 569–578.

Dickson, B., Sprenger, F., Morrison, D., and Hafen, E. (1992). Raf functions downstream of Ras1 in the Sevenless signal transduction pathway. *Nature* 360, 600–603.

Dickson, B., and Hafen, E. (1993). Genetic dissection of eye development in *Drosophila*. *In* ''The Development of *Drosophila melanogaster*. (M. Bate, and A. Martinez Ariaz, Eds.), pp. 1327–1362. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Dickson, B., and Hafen, E. (1994). Genetics of signal transduction in invertebrates. *Curr. Opin. Genet. Dev.* 4, 64–70.

Doyle, H. J., and Bishop, J. M. (1993). Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the sevenless and EGF-R pathways in *Drosophila*. *Genes Dev.* 7, 633–646.

Fehon, R. G., Kooh, P. J., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. T., and Artavanis-Tsakonas, S. (1990). Molecular interac-

tions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in Drosophila. *Cell* 61, 523–534.

Fortini, M. E., Simon, M. A., and Rubin, G. M. (1992). Signaling by the *sevenless* protein tyrosine kinase is mimicked by Ras1 activation. *Nature* 355, 559–561.

Freeman, M. (1994a). The *spitz* gene is required for photoreceptor determination in the *Drosophila* eye where it interacts with the EGF receptor. *Mech. Dev.* 48, 25–33.

Freeman, M. (1994b). Misexpression of the *Drosophila argos* gene, a secreted regulator of cell determination. *Development* 120, 2297–2340.

Freeman, M., Klämbt, C., Goodman, C. S., and Rubin, G. M. (1992). The *argos* gene encodes a diffusible factor that regulates cell fate decisions in the *Drosophila* eye. *Cell* 69, 963–975.

Gaul, U., Mardon, G., and Rubin, G. M. (1992). A putative Ras GTPase activating protein acts as a negative regulator of signaling by the sevenless receptor tyrosine kinase. *Cell* 68, 1007–1019.

Hafen, E., Basler, K., Edstroem, J. E., and Rubin, G. M. (1987). *sevenless*, a cell-specific homeotic gene of *Drosophila*, encodes a putative transmembrane receptor with a tyrosine kinase domain. *Science* 236, 55–63.

Heberlein, U., Hariharan I. K., and Rubin, G. M. (1993). *Star* is required for neuronal differentiation in the *Drosophila* retina and displays dosage-sensitive interactions with ras1. *Dev. Biol.* 160, 51–63.

Heitzler, P., and Simpson, P. (1991). The choice of cell fate in the epidermis of *Drosophila*. *Cell* 64, 1083–1092.

Hou, X. S., Chou, T.-B., Melnick, M. B., and Perrimon, N. (1995). The Torso receptor tyrosine kinase can activate Raf in a Ras-independent pathway. *Cell* 81, 63–71.

Hsu, J., and Perrimon, N. (1994). A temperature-sensitive MEK mutation demonstrates the conservation of the signaling pathways activated by receptor tyrosine kinases. *Genes Dev.* 8, 2176–2187.

Klingler, M., Erdélyi, M., Szabad, J., and Nüsslein-Volhard, C. (1988). Function of torso in determining the terminal anlagen of the *Drosophila* embryo. *Nature* 335, 275–277.

Kolodkin, A. L., Pickup, A. M., Lin, D. M., Goodman, C. S., and Banerjee, U. (1994). Characterizing of *Star* and its interactions with sevenless and EGF receptor during photoreceptor cell development in *Drosophila*. *Development* 120, 1731–1745.

Kretzschmar, D., Brunner, A., Wiersdorff, V., Pflugfelder, G. O., Heisenberg, M., and Schneuwly, S. (1992). *ginat lens*, a gene involved in cell determination and axonal guidance in the visual system of *Drosophila* melanogaster. *EMBO J.* 11, 2531–2539.

Lu, X., Melnick, M. B., Hsu, J.-C., and Perrimon, N. (1994). Genetic and molecular analyses of mutations involved in *Drosophila raf* signal transduction. *EMBO J.* 13, 2592–2599.

Nishida, Y., Hata, M., Ayaki, T., Ryo, H., Yamagata, M., Shimizu, K., and Nishizuka, Y. (1988). Proliferation of both somatic and germ cells is affected in the *Drosophila* mutants of *raf* proto-oncogene. *EMBO J.* 7, 775–781.

Okabe, M., Sawamoto, K., and Okano, H. (1996). The function of *Drosophila argos* gene product in the development of embryonic chordotonal organs. *Dev. Biol.* 175, 37–49.

Okano, H., Hayashi, S., Tanimura, T., Sawamoto, K., Yoshikawa, S., Watanabe, J., Iwasaki, M., Hirose, S., Mikoshiba, K., and Montell, C. (1992). Regulation of *Drosophila* neural development by a putative secreted protein. *Differentiation* 52, 1–11.

Olivier, J. P., Raabe, T., Henkemeyer, M., Dickson, B., Mbamalu, G., Margolis, B., Schlessinger, J., Hafen, E., and Pawson, T. (1993). A *Drosophila* SH2-SH3 adaptor protein implicated in coupling the Sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. *Cell* 73, 179–191.

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, P., and Artavanis-Tsakonas, S. (1991). Specific EGF repeats of Notch mediate interactions with Delta and Serrate: Implications for Notch as a multifunctional receptor. *Cell* 67, 687–699.

Rogge, R. D., Kariovich, C. A., and Banerjee, U. (1991). Genetic dissection of a neurodevelopmental pathway: Son of sevenless functions downstream of the sevenless and EGF receptor tyrosine kinases. *Cell* 64, 39–48.

Sawamoto, K., Okano, H., Kobayakawa, Y., Hayashi, S., Mikoshiba, K., and Tanimura, T. (1994). The function of *argos* in regulating cell fate decisions during *Drosophila* eye and wing vein development. *Dev. Biol.* 164, 267–276.

Sawamoto, K., Okabe, M., Tanimura, T., Hayashi, S., Mikoshiba, K., and Okano, H. (1996). *argos* is required for projection of photoreceptor axons during optic lobe development in *Drosophila*. *Dev. Dyn.* 205, 162–171.

Schüpbach, T. (1987). Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of the egg shell and embryo in *Drosophila* melanogaster. *Cell* 49, 699–707.

Schweitzer, R., Howes, R., Smith, R., Shilo, B.-Z., and Freeman, M. (1995a). Inhibition fo *Drosophila* EGF receptor activation by the secreted protein Argos. *Nature* 376, 699–702.

Schweitzer, R., Shaharabany, M., Seger, R., and Shilo, B.-Z. (1995b). Secreted spitz triggers the DER signaling pathway and is a limiting component in embryonic ventral ectoderm determination. *Genes Dev.* 9, 1518–1529.

Simon, M. A., Bowtell, D. D., Dodson, G. S., Laverty, T. R., and Rubin, G. M. (1991). Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell* 67, 701–716.

Simon, M. A., Dodson, G. S., and Rubin, G. M. (1993). An SH3-SH2-SH3 protein is required for p21Ras1 activation and binds to sevenless and Sos proteins in vitro. *Cell* 73, 169–177.

Sprenger, F., Stevens, L. M., and Nüsslein-Volhard, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature* 338, 478–483.

Sturtevant, M. A., Roark, M., and Bier, E. (1993) The *Drosophila rhomboid* gene mediates localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. *Genes Dev.* 7, 961–973.

Tio, M., Ma, C., and Moses, K. (1994). spitz, a *Drosophila* homolog of transforming growth factor-α, is required in the founding photoreceptor cells of the compound eye facets. *Mech. Dev.* 48, 13–23.

Tomlinson, A., and Ready, D. F. (1986). *Sevenless*, a cell-specific homeotic mutation of the *Drosophila* eye. *Science* 231, 400–402.

Tsuda, L., Inoue, Y. H., Yoo, M., Mizuno, M., Hata, M., Lim, Y., Adachi-Yamada, T., Ryo, H., Masamune, Y., and Nishida, Y. (1993). A protein kinase similar to MAP kinase activator acts downstream of the raf kinase in *Drosophila*. *Cell* 72, 407–414.

Wemmer, T., and Klämbt, C. (1995). A genetic analysis of the *Drosophila* closely linked interacting genes *bulge*, *argos* and *soba*. *Genetics* 140, 629–641.

Xu, T., and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 117, 1223–1237.

Received for publication December 7, 1995
Accepted June 17, 1996